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EXTRACTION AND ANALYSIS OF HEXACHLOROXANTHENE USING MODIFIED U.S. EPA METHOD 1613, REVISION B PROCEDURES

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Introduction

The production of hexachlorophene yields 1,2,4,5,7,8-hexachloro-9H-xanthene (HCX) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as by-products. HCX has been indicated as a marker usable for tracing TCDD contamination back to hexachlorophene production. [1,2]

There has been a growing interest in being able to test samples from contaminated sites for HCX along with the 17 polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF). Previous efforts to quantitatively determine HCX and PCDD/PCDF using U.S. EPA Method 1613, Revision B (1613B)[3] have been limited because this method does not specify exact ion masses for HCX[2]. Other approaches have used a single point calibration for generating HCX response factors relative to native TCDD[1] rather than a multi-point calibration against an internal standard. The work described here attempts to resolve these limitations and shows the results of extracting and analyzing tissue and sediment/soil samples for HCX concurrently with the 17 PCDD/PCDF using 1613B modified specifically for determination of HCX along with a multi-point calibration for the quantitation of HCX.

Methods and Materials

HCX Standard:

A stock standard of HCX was obtained from Cambridge Isotope Laboratories, Inc. (CIL). In the production of this standard, CIL discovered that HCX showed stability problems when in contact with solvents traditionally used for PCDD/PCDF analysis. Because of these stability issues, the HCX standard was kept in residue form for long term storage during this study.

Table 1. Degradation of HCX in Solution

Solvent	Percent Degradation after 1 Month
Nonane	50%
Nonane with a stabilizer	10%
Methylene Chloride	10%
Trichloroethane	10%

Qualitative Determination of HCX:

An HCX standard was prepared and analyzed by gas chromatography/high resolution mass spectrometry (GC/HRMS) in full scan mode to determine the ion masses to be used for identification of the analyte. HCX ion masses of 387.8365 and 389.8325 were added to the selected ion monitoring function for hexa-chlorinated compounds in 1613B. $^{13}C_{12}$-1,2,3,7,8,9-HxCDF was used as the internal standard for quantifying HCX since a labeled standard for HCX was not available and because it was the standard which eluted most closely to HCX. With these modifications one analytical run could be used for the determination of both the 17 PCDD/PCDF and HCX.

Initial Calibration for HCX:
Eight HCX calibration standards were analyzed by HRMS on a VG-Autospec (Micromass) using a DB-5 (60m x 0.32mm x 0.25 μm) column (J&W Scientific). HCX was not detectable in the two lowest concentration calibration standards and so only calibration points CS-3 through CS-8 were used. There was a significant difference in response factors generated from CS-3 through CS-5 as compared to CS-6 through CS-8. Because of this response difference, the six calibration standards were divided into two curves (3 calibration standards in each) in order to get an average response factor with relative standard deviation (RSD) below 50%. Table 2 shows the curve levels, response factors (RF), average response factors, standard deviations, RSD, and ion ratios generated for HCX from this calibration. Criteria for selecting which curve to use for quantitation are discussed under "Quantitation of HCX" below.

Table 2. HCX Initial Calibration Curves Summary and Ion Ratios

Calibration Standard	Calibration Level (ng/mL)	HCX Curve 1 (RF)	HCX Curve 2 (RF)	All 6 Calibration Points (RF)	Ion Ratios
CS-1	1.25	ND		ND	ND
CS-2	2.5	ND		ND	ND
CS-3	10	0.12		0.12	1.33
CS-4	50	0.15		0.15	1.27
CS-5	200	0.19		0.19	1.21
CS-6	1000		0.07	0.07	1.20
CS-7	2000		0.04	0.04	1.20
CS-8	4000		0.03	0.03	1.22
Average RF		0.15	0.05	0.10	
Standard Deviation		0.04	0.02	0.06	
%RSD		23	45	64	
Average Ion Ratio					1.24

Sample Preparation:
Sediment and tissue samples were prepared by adding PCDD/PCDF labeled internal standard (CIL EDF-8999-4) and native PCDD/PCDF (CIL EDF-7999) at the levels specified in 1613B. The samples were also spiked with 4000 pg of HCX. The samples were extracted using a Dionex Accelerated Solvent Extractor (ASE) with dichloromethane:hexane (50:50) for the tissue extraction solvent and toluene for the sediments. The sample extracts were spiked with 2,3,7,8-TCDD$^{37}Cl_4$ cleanup standard (CIL EDF-6999) and processed through general cleanup procedures found in 1613B. Extracts were concentrated to a 20 μL final volume and a recovery standard of 1,2,3,4-TCDD$^{13}C_{12}$ and 1,2,3,7,8,9-HxCDD$^{13}C_{12}$ (CIL EDF-5999) was added.

Analysis of Extracts:
Samples extracts were analyzed on a VG-Autospec HRMS using the same conditions and method as the HCX initial calibration curve discussed above. A 1 μL injection volume was used.

Quantitation of HCX:
Concentrations of HCX detected in samples were calculated using $^{13}C_{12}$-1,2,3,7,8,9-HxCDF as an internal standard and either the RF from HCX Curve 1 or HCX Curve 2. The break point between Curve 1 and Curve 2 was arbitrarily chosen as the midpoint between CS-5 and CS-6. The following criteria were used to determine whether the RF from Curve 1 or Curve 2 should be used. The peak areas for the two HCX ion masses were summed, if the sum of the areas for the HCX ions in the sample was below the average sum of areas for HCX in CS-5 and CS-6, then the RF from HCX Curve 1 was used. If the sum of the areas for the HCX ions in the samples was above the average sum of areas for HCX in CS-5 and CS-6, then the RF from HCX Curve 2 was used. If the sum of the areas for the HCX ions in the sample happened to be equal to the average sum of areas for HCX in CS-5 and CS-6, then a judgment call was made on which RF to use.

Results and Discussion
The percent recoveries for HCX in the spiked samples are shown in Table 3 and are listed by matrix. Results for samples in which the HCX was not spiked greater than 10 times the background amount are not included. For comparison, the 1,2,3,7,8,9-HxCDF recoveries are also included.

Table 3. Percent Recoveries of HCX and 1,2,3,7,8,9-HxCDF in Spiked Samples

Tissue HCX (% Recovery)	Tissue 1,2,3,7,8,9-HxCDF (% Recovery)	Sediment/Soil HCX (% Recovery)	Sediment/Soil 1,2,3,7,8,9-HxCDF (% Recovery)
99	96	111	97
64	97	101	98
126	86	43	90
163	86	109	96
95	88	--	--
112	88	--	--
64	95	--	--
132	92	--	--
93	93	--	--
73	91	--	--
129	93	--	--
181	93	--	--
91	78	--	--
60	80	--	--
85	99	--	--
140	103	--	--
112	97	--	--
107	100	--	--
107*	**92***	**91***	**95***

* = average percent recovery

These results show that HCX survives the 1613B extraction and cleanup procedure with recoveries comparable to 1,2,3,7,8,9-HxCDF. With the addition of HCX ions to the 1613B HRMS parameters, HCX can be analyzed concurrently with PCDD/PCDF. While this modified method incorporates a multi-point calibration for HCX, the calibration can be improved upon by better evaluating the linear ranges of the calibration curves and establishing an actual breakpoint between the two curves rather than making an arbitrary selection.

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